REMARKS

Reconsideration is requested.

Claim 7 has been added and finds support throughout the specification. Claim 7 defines further aspects of the disclosed invention relating to the promoter. Support for the amendment may be found, for example, on page 9, lines 35-36 of the specification. No new matter has been added. Claims 1 and 3-7 are pending.

The Section 103 rejection of claims 1 and 3-6 over Beck (Mechanism of Development, 1999, 88:221-227), Witte (Microscopy Research and Technique, 2001, 55:122-145) and Bartel (Anat Embryol, 2000, 202:55-65), is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing comments.

The applicants submit that the publication of Beck et al., entitled "Gut specific expression using mammalian promoters in transgenic *Xenopus laevis*", teaches a transgenic *Xenopus* comprising GFP under the control of mammalian tissue specific promoters, wherein the promoters drive specific GFP expression in different parts of the gut. As acknowledged on page 3 of the Office Action dated February 20, 2009, Beck et al. do not specifically teach making transgenic *Xenopus* comprising GFP under the control of promoters specific for expression within the lymphatic vessels (as specified in claims 1, 3 and 4 of the present application), nor do they teach using this transgenic Xenopus to visualize the lymphatic vessel system (as defined in claim 5) or to screen for compounds capable of modulating lymphatic vessel development (as defined in claim 6).

The applicants submit that Witte et al. teach that the lymphatic vessel development is poorly understood and describe the use of experimental mammalian models to elucidate the mechanism of lymphatic vessel development. The applicants submit that Bartel et al. teach that *Xenopus* has lymphatic vessels.

With regard to the teachings of Witte et al., the Examiner states that these authors *"*teach the necessity to identify agents able to modulate lymphatic vessel growth (p. 138, column 1)*"*. See page 4 of the Office Action. On page 6 of the Office Action, it is even stated that *"*Witte et al. teach using mammalian models to screen for agents capable of modulating lymphatic vessel growth*"*.

The applicants respectfully disagree with the Examiner's interpretations of the cited art and consideration of the following in this regard is requested.

On page 138, column 1, Witte et al. only discuss refined imaging methods (MRI, NMR, videomicroscopy) to study lymphatic structures. Also in the section on experimental animal models on page 127-128 of Witte et al., no screening model has been proposed: the models serve as basis for elucidating the mechanisms of lymphatic development. See e.g. the introduction to this section, on page 127, column 1:

> "A variety of experimental preparations either newly developed or worthy of being revisited with modern techniques hold promise <u>to elucidate the mechanisms of lymphvasculogenesis/lymphangiogenesis</u> and their disorders, whether exuberant (compensatory) or defective (causative)." (emphasis added)

The word 'screen' occurs once in the section on animal models, on page 128, column 1 however this is in a different context. Specifically, this section does not refer to using animal models to screen for agents capable of modulating lymphatic vessel

development, but relates to screening humans for the presence of infection by looking at the presence of live adult worms:

> "The earliest clinically detectable abnormality in filariasis is hindlimb lymphatic dilatation and collateralization related to nests of live adult worms thrashing about inside hindlimb lymphatics. These lymphangiogenic events can not only be seen invasively (Case et al., 1992a) and imaged noninvasively (Witte et al., 1988), but also can be recreated in vitro. This early lymphatic abnormality has been used to screen for productive (microfilaremic) infection (Witte et al., 1993) and also exploited to follow the efficacy of therapeutic intervention in patients in endemic areas such as northeastern Brazil (Amaral et al., 1994) and in Southern India (Suresch et al., 1997)" (page 128 of Witte et al., column 1)

It is noted that the cited Witte et al., 1993 reference (Arch Intern Med 153:737-744) relates strictly to screening patients for particular symptoms, not to screening compounds for their effect in an animal model. Thus, the Examiner's assertion that Witte et al. 'teach the necessity to identify agents able to modulate lymphatic vessel growth' and that Witte et al. 'teach using mammalian models to screen for agents capable of modulating lymphatic vessel growth' is incorrect and appears based on a misinterpretation of the teachings of Witte et al. As there is no single indication or suggestion in Witte et al. to screen the models for compounds capable of modulating lymphatic vessel development, the Examiner is requested to withdraw the Section 103 rejection.

Moreover, it is noted that, based on the teachings of Witte et al., one of ordinary skill in the art would not have been inclined to have combined the reference with Beck et al., as suggested by the Examiner. In the introduction of Witte et al., it is believed to

be clear that amphibians do not have lymph nodes, as these appear only later in evolution:

> "As species emerged from an aquatic environment, lymph nodes first made their appearance in birds and then in mammals, interspersed between lymph collectors and structurally pose a potential site of restriction to the free percolating flow of lymph. Further refined in mammals, including man, are well-defined lymphatic segments termed "lymphangions" situated between innumerable intraluminal valves and capable of rhythmic intrinsic contractions critical to propulsion of lymph." (page 122, paragraph spanning column 1 and 2; emphasis added)

It is repeatedly emphasized in the Witte et al. publication that these lymph nodes (or, in mammals, also lymphangions, the segments between the valves) are an important factor in trafficking of immune cells, and that defects in the transport system between two valves gives rise to inflammation and - localized - edema.

> "In conjunction with interspersed lymph nodes and lymphoid organs, the lymphatic vasculature also acts as a conduit for trafficking immune cell populations." (Abstract, page 122)

> "Because lymph propulsion depends predominantly on intrinsic truncal contraction, once lymphatics become obstructed truncal contractions initially quicken but then intraluminal valves gradually become incompetent and hydrostatic pressure in the draining tissue watersheds and lymphatics rises as intrinsic truncal contractions fail to expel lymph completely." (page 135, 2nd column)

> "Functionally, obstruction occurs at the deep truncal or lymph node level." (page 137, 1st column)

Witte et al. conclude that the dynamic structural changes of the lymph vasculature can be imaged, even in small animal models –again stressing the importance of the nodes (here also indicated as 'lymphatic collectors'):

> "Magnetic resonance imaging provides further information on soft tissue changes in chemical composition (water, fibrous tissue and fat) and sites of pooling of lymph/edema fluid and ectatic lymphatic collectors. Experimentally, addition of magnetic contrast has produced the first lymphangiomagnetograms. These techniques (dye injection, LAS, MRI) have even been adapted to small animals, with major improvements in high-resolution imaging of lymphatic channels and nodes, making this once invisible system accessible to study even in 2-g mouse pups." (page 137, 2nd column, references omitted, emphasis added)

Considering that the Witte et al. reference primarily relates to lymphedema and the underlying lymphatic failure; that the authors teach that lymphedema is the result of a lymphatic obstruction, and that this obstruction occurs 'at the deep truncal or lymph node level', an ordinarily skilled person, if looking for further models, would have looked for models able to recapitulate these features, i.e. models with contractile truncal walls (a feature of lymphangions) and/or lymph nodes. The very techniques proposed by Witte et al. (lymphangiography (LAG), lymphangioscintigraphy (LAS), or lymphangiomagnetography – see Witte et al., page 136-138) aim to visualize lymphangions and lymph nodes.

Accordingly, Witte et al. describe several mammalian models. As for small animal models, Witte teaches using mouse pups, in which "high-resolution imaging of lymphatic channels and nodes" is also possible.

An ordinarily skilled person would thus not have been inclined or motivated from the cited art to have used a lower vertebrate model, as it is taught that amphibians and reptiles do not have lymph nodes (these only emerged in birds), nor contractile lymphatic truncal walls/lymphangions (these are a feature of mammals). Consequently,

it appears from Witte et al. that amphibians are an unsuitable model system for studying lymphedema and the underlying lymphangiogenesis, as there is no indication that they possess a system for lymph transport with 'potential sites of restriction to the free percolating flow of lymph', a feature necessary for localized obstruction of lymph flow and the resulting localized edema, and as is evident from Witte et al. only present in birds and mammals.

Further combination with Bartel et al. does not overcome the above-noted deficiencies, as Bartel et al. suggest only the presence of lymphatic vessels – they do not disclose anything about the functionality of the vessels or about the presence of other features of the lymphatic system.

The present application is the first to show a functional lymphatic system in *Xenopus* tadpoles, including lymphatic sacs (which may serve as lymphatic collectors).

The Examiner asserts that the claimed invention would have allegedly been obvious from the cited combination of references of Beck et al. and Witte et al.. Specifically, the Examiner is understood to believe that Witte et al. allegedly teach using mammalian models to screen for agents capable of modulating lymphatic vessel growth; that Beck et al. allegedly provide the motivation to use transgenic *Xenopus* and not the transgenic mice of Witte et al.; and that Bartel et al. and Witte et al. allegedly teach the existence of lymphatic vessels and lymph heart in *Xenopus* tadpoles.

This reasoning is, however, submitted to be incorrect as the starting premise is incorrect: Witte et al. do not teach the necessity to identify agents able to modulate lymphatic vessel growth. This is not remedied by Beck et al., who are silent about a

model of the lymphatic system, as well as about a compound screening method. Moreover, although Beck et al. mention several advantages of the *Xenopus* system, this does not imply that the system can automatically or predictably be used for other applications. In this regard, Witte et al. mention that amphibians lack lymph nodes and lymphatic vessels with intrinsic contractile truncal walls; which are the sites where obstruction (and the resulting lymphedema) occurs. Thus, even when combining Witte et al. with Beck et al., one would not have had a reasonable expectation of success to obtain an animal with functional lymphatic vessels, lymphatic sacs and a lymphatic heart, as required by the claims of the present application, particularly since all references are silent on the presence of lymphatic sacs. Moreover, it is clear from Witte et al. that all models used for studying lymphangiogenesis or the resulting lymphedema have lymph nodes and/or contractile truncal walls in the lymph vessels and that this is indeed necessary for functional obstruction to occur. Based on the teachings of Witte et al., an ordinarily skilled person would have used mice or other mammals as (small) animal models, because they recapitulate the necessary features. Stating that the ordinarily skilled person would have combined Witte et al. with Beck et al. to result in a transgenic *Xenopus* model ignores part of the teaching of Witte et al., and is thus based on an improper interpretation of Witte et al. or on inappropriate use of hindsight.

At the time the invention was made, an ordinarily skilled person would not have known, for example, that lymph sacs were present in *Xenopus* and would not have combined Witte et al. with Beck et al. because lymph nodes or similar structures are absent in *Xenopus* according to Witte et al. Moreover, the ordinarily skilled person

would not have used any animal model in a method of screening based on these two references, as there is no teaching or suggestion in either reference to screen for agents able to modulate vessel growth.

For all the above reasons, the applicants submit that the claimed invention would not have been obvious over the cited combination of art and withdrawal of the Section 103 rejection is requested.

With regard to new claim 7, the same considerations apply. Applicants furthermore submit that Beck et al. provide no indication to use *Xenopus* promoters. Indeed, Beck et al. state that "only a small number of Xenopus promoters have been characterized in live embryos" (page 221, 1st column), and that "the fact that mammalian promoters can be used is of considerable importance for designing experiments on the development of Xenopus itself" (page 225, 2nd column, 3rd full paragraph) as this "will avoid the need for the cloning of endogenous promoters for misexpression studies" (ibidem).

The claims are submitted to be patentable over the cited combinations of art. Withdrawal of the Section 103 rejections is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested. The Examiner is requested to contact the undersigned, preferably by telephone, in the event anything further is required.

Respectfully submitted,

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